B9 Cont from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-inosinic acid.

B10 8. (Amended) The process according to claim 20, wherein the non-sugar carbon source is acetic acid or acetate.

B11 20. (New) The process according to claim 7, wherein expression of the enzyme is induced by change of carbon source from a sugar carbon source to a non-sugar carbon source.

REMARKS

Claims 3 and 4 have been amended, and claim 20 presented in order to recite a preferred embodiment of the present invention with the specificity required by statute. Additionally, Claim 8 has been amended to better depend from its antecedent claim. The specification has been amended in order to correct any inadvertent typographical errors noted therein. Accordingly, no new matter has been added.

Regarding an initial formal matter, the Examiner noted the citation to EP 0 236 716 A but declined to consider it pending an explanation of the relevance of a patent drawn to electrical connectors. Applicants agree the citation is curious, but was included because it was cited by the Hague as being particularly relevant if taken alone to claims 10 and 11 . See the July 5, 2000 European Search Report enclosed with the August 23, 2000 Information Disclosure Statement. What the European Examiner was thinking, neither Applicants nor the undersigned can say.

Regarding another formal matter, the Examiner has required submission of a Statement under 37 C.F.R. §1.821. In response, such Statement is attached. Accordingly, this requirement is met.

The Examiner had requested that Applicants affirm their provisional election to prosecute the invention of: Group I (Claims 1-8), drawn to processes for producing purine nucleotides. By the above cancellation of nonelected claims 9-19, this provisional election is hereby affirmed.

The Abstract has been objected to for the reasons noted. In response, Applicants have submitted an amended Abstract in conformity with the Examiner's kind suggestions. Accordingly, this rejection is mooted.

Claims 1-8 are rejected under 35 U.S.C. §112, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner's basis for this rejection are set forth in paragraphs 12-15. In particular, the Examiner contends the term "precursor" in claim 1 is indefinite since virtually any compound can be a precursor when pathways to convert the "precursor" into the product are undefined.

Respectfully submitted, this rejection is not well understood since the phrase "precursor of the purine nucleotide" is well discussed in the specification. Applicants are entitled to be their own lexicographer and have shown that XMP, guanosine, inosine and adenosine" plainly function as examples of the precursors of purine nucleotides in the specification. Therefore, it is easily understood by those of ordinary skill herein that the precursors are not "any compound" but are those materials on the purine nucleotide synthetic pathway.

Claims 1-8 are rejected under 35 U.S.C. 112, second paragraph for the reason that the terms "carrying" and "introduced" are not specific terms of art. In response, claim 1 has been amended as kindly suggested by the Examiner.

Claims 3-4 are rejected under 35 U.S.C. 112, second paragraph for the reason that the enzyme name "inosine-guanosine kinase or phosphatase" is unclear. In

order to clarify this subject matter,"phosphatase" has been amended to read "acid phosphatase" as also kindly suggested by the Examiner.

Claim 8 is rejected under 35 U.S.C. 112, second paragraph for the reason that the terms "acetic acid" and "acetate" are said to be redundant. In response, Applicants respectfully wish to explain that such are clearly different compounds and there is no redundancy between them since "acetic acid" is a kind of organic acid and "acetate" is a salt of acetic acid with a metal.

Claims 7-8 are rejected under 35 U.S.C. 112, first paragraph. The Examiner states that the instant claims are drawn to processes which use expression systems induced by a rise in pH and/or a rise in osmotic pressure whereas Applicants have only described promoters which function via a rise in temperature, PL promoter, and via acetate, isocitrate lyase promoter.

In response, Applicants wish to invite the Examiner's attention to specification page 13, line 35 to page 14, line 3, wherein pRK248cIts (which contains thePL promoter/cI857 repressor gene and is induced at a high pH, see Gene, 97), and pOSEX2 (which contains the proU expression-regulating region and is induced under a high osmotic pressure) are described as examples of the vectors which may be utilized in the present invention.

Claims 1-2, 5 and 7-8 stand rejected under 35 U.S.C. 102 (b) as anticipated by Fujio et al. (Biosci. Bottech. Biochem. 61(5), 840-845, 1997). The Examiner states that the instant claims are simply drawn to methods of making GMP in *Escherichia* using a temperature-sensitive induction expression system of recombinant GMP synthetase, whereas Fujio teaches a method of production of GMP using *E. coli* transformed with a gene for GMP synthetase (XMP aminase) under the control of the temperature-sensitivePL promoter. Therefore, since the Examiner contends that since *E. coli* inherently contain the precursor XMP, and Fujio teach industrial production of GMP (thus inherently containing

a recovery step), accordingly that the claims are anticipated. This rejection is respectfully traversed.

The process of the present invention is characterized by culturing a recombinant microorganism transformed with a gene which can express an enzyme capable of converting a precursor (for example, XMP) to purine nucleotide (for example, GMP) in a host microorganism having the ability to produce the precursor, and after incubation, converting the precursor to the purine nucleotide inducing the expression of the gene in the microorganism.

As a result of the microorganism etc., the present invention is able to produce a purine nucleotide in which fermentation to produce a precursor of the nucleotide and the reaction to convert said precursor into the nucleotide can be carried out successively using only one microorganism in one fermentor. Neither the process of the present invention nor the advantages obtained thereby (e.g., using a single fermentor etc.) are either taught or suggested by the prior art.

Fujio relates to producing GMP using a mutant of *Corynebacterium ammoniagenes* and *E. coli* transformed with a GMP synthetase gene. In Fujio, the mutant of *Corynebacterium ammoniagenes* and the *E. coli* transformant are cultured separately, and after the culturing, the culture broths are combined to convert XMP to GMP. The method of Fujio et al. thus needs <u>two</u> microorganism strains and two fermentors.

As explained above, the process of the present invention is characterized in that it can be conducted using one microorganism strain. The method of Fujio differs in kind, not least because it utilizes a coupling reaction between two disparate kind of strains of organisms. It is clear that the use of two kind of strains is essential in the method of Fujio whereas the pending claims recite inducing expression of the appropriate enzyme in the cultured microorganism. Therefore, even if, arguendo, *Escherichia coli* inherently contains XMP, such does <u>not</u> mean that the nucleotide production can be conducted

efficiently in *Escherichia coli* alone. For all these reasons, the process of the present invention well distinguishes the method of Fujio.

Further, simply in order to still further distinguish the prior art, Applicants wish to point out that even the elements of the present invention are not contained by Fujio. In this regard, the process of the present invention enables efficient production of purine nucleotides by controlling when a gene coding for the enzyme capable of synthesizing the purine nucleotide from a precursor is expressed using a plasmid containing an inducible factor such asPL promoter. Pending Claim 1 defines the elements of the invention that the precursor is accumulated and then the gene is expressed upon induction to convert the precursor to the purine nucleotide.

Fujio does not disclose or suggest any of these elements but merely state that it is possible to enhance the XMP aminase activity of *E. coli* by using a gene containingPL promoter and to convert XMP to GMP by mixing the culture broth of *E. coli* and XMP-fermentation broth of *C. ammoniagenes*. In the Discussion, Fujio describes

> "We have obtained the XMP aminase-overproducing strain MP347/pPLA66 using the PL-ATG vector... "ThePL promoter system is strictly regulated by temperature, therefore we could obtain a higher activity strain than MM294/pXAR33."

These descriptions show thatPL promoter is used for the purpose of enhancing XMP aminase activity in Fujio.

Since Fujio does not comprise time-control of the production of XMP and the expression of XMP aminase gene in *E. coli* strain, the XMP can not be efficiently converted to GMP even if XMP is inherently contained in *E. coli*.

Claims 1 and 3-6 are rejected under 35 U.S.C. 102(b) as being anticipated by Usuda et al. (EP0816491). Usada is cited as showing that purified precursors such as inosine and guanosine are converted to IMP and GMP using a *Corynebacterium* transformed with inosine-guanosine kinase gene.

As mentioned above, the process of the present invention is a process for producing a purine nucleotide in which fermentation to produce a precursor of the nucleotide and reaction to convert said precursor into the nucleotide can be carried out successively using but one microorganism in one fermentor. In the process of the present invention, the substrate of the nucleotide, i.e. the precursor of the nucleotide is provided by the culturing of the recombinant microorganism and it is not necessary to supply a purified precursor. Accordingly, the process of the present invention is quite different from Usuda.

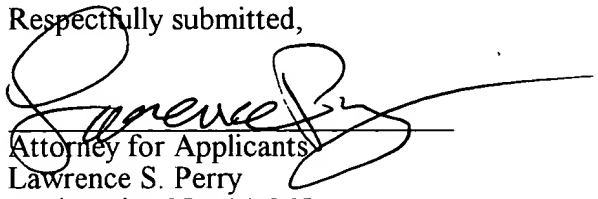
Claims 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Usuda in view of Katsumata et al. (US 5,439,822). As mentioned above, the processes of claims 7 and 8 are different from the method of Usuda in the method of providing precursors of the nucleotides. This deficiency is not addressed by the secondary reference which relates only to the ICL promoter.

In view of the above amendments and remarks, Applicants submit that all of the Examiner's concerns are now overcome and the claims are now in allowable condition. Accordingly, reconsideration and allowance of this application is earnestly solicited.

Claims 1-8 remain presented for continued prosecution.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should be directed to our below listed address.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

1. (Twice Amended) A process for producing a purine nucleotide which comprises:

culturing in a medium a microorganism having the ability to produce a precursor of the purine nucleotide and [carrying an introduced] transformed with DNA which can express an enzyme capable of synthesizing the purine nucleotide from said precursor upon induction;

allowing said precursor of the purine nucleotide to accumulate in the culture;

inducing the expression of the enzyme capable of synthesizing the purine nucleotide from said precursor;

allowing the purine nucleotide formed from said precursor by said expressed enzyme to accumulate in said culture; and

recovering said purine nucleotide therefrom

3. (Amended) The process according to claim 1, wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-guanylic acid.

4. (Amended) The process according to Claim 1, wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide

from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-inosinic acid.

8. (Amended) The process according to claim [7] 20, wherein the non-sugar carbon source is acetic acid or acetate.

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO SPECIFICATION

The paragraph starting at page 3, line 25 and ending at page 4, line 4 has been amended as follows:

As for the phosphorylation, methods using phosphotransferase, kinase and phosphatase are known. In particular, the reaction utilizing kinase or phosphatase has been studied as an efficient method. For example, there have been developed a process for producing a 5'-nucleotide by the use of an *Escherichia coli* strain carrying a gene encoding inosine-guanosine kinase of *Escherichia coli* (W091/08286), a process for producing a 5'-nucleotide by the use of a *Corynebacterium ammoniagenes* strain carrying a gene encoding inosine-guanosine kinase of *Exiguobacterium acetylicum* (W096/30501), and a process for producing a 5'-nucleotide by the use of an *Escherichia coli* strain carrying a gene prepared imparting a random mutation to the acid phosphatase gene of *Morganella morganii* (Japanese Published Unexamined Patent Application No. 37785/97, Japanese Published Unexamined Patent Application No. 201481/98).

The paragraphs at page 7, lines 11 through 20 have been amended as follows:

(3) The process according to the above (1), wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-guanylic acid.

(4) The process according to the above (1), wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-inosinic acid.

The paragraphs at page 8, lines 21 through 30 have been amended to read as follows:

(15) The microorganism according to the above (13), wherein the precursor of the purine nucleotide is guanosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-guanylic acid.

(16) The microorganism according to the above (13), wherein the precursor of the purine nucleotide is inosine, the enzyme capable of synthesizing the purine nucleotide from said precursor is inosine-guanosine kinase or acid phosphatase, and the purine nucleotide is 5'-inosinic acid.

The paragraph starting at page 11, line 31 and ending at page 12, line 1 has been amended to read as follows:

As the enzyme capable of synthesizing a purine nucleotide from its precursor to be used in the present invention, any enzyme capable of synthesizing a purine nucleotide from its precursor can be used, and suitable examples include XMP aminase, inosine-guanosine kinase, acid phosphatase and adenylate kinase.

The paragraph at page 12, lines 12-15 has been amended to read as follows:

Genes encoding acid phosphatase include those derived from *Morganella morganii* (Japanese Published Unexamined Patent Application No. 37785/97, Japanese Published Unexamined Patent Application No. 201481/98), etc.

The paragraph starting at page 12, line 30 and ending at page 13, line 4 has been amended to read as follows:

In the case of the gene encoding inosine-guanosine kinase, primers are synthesized based on the sequences at both ends of the sequence of an inosine-guanosine kinase structural gene, and the inosine-guanosine kinase structural gene can be obtained by the PCR method using the prepared primers and the *Escherichia coli* chromosomal DNA or the *Exiguobacterium acetylicum* chromosomal DNA. Similarly, by the use of the PCR method, a acid phosphatase structural gene can be obtained from the *Morganella morganii* chromosomal DNA and an adenylate kinase structural gene can be obtained from the *Saccharomyces cerevisiae* chromosomal DNA.

Page 40 has been amended to read as follows:

ABSTRACT OF THE DISCLOSURE

Purine nucleotides are produced by culturing [in a medium] a microorganism having the ability to produce a precursor of the purine nucleotide and carrying an introduced DNA which can express an enzyme capable of synthesizing the

purine nucleotide from [said] the precursor upon induction; allowing [said precursor of] the purine nucleotide precursor to accumulate in the culture; inducing the expression of the enzyme [capable of synthesizing the purine nucleotide from said precursor and expressing the enzyme]; allowing the purine nucleotide formed [from the precursor] to accumulate in the culture; and recovering the purine nucleotide [therefrom]. Suitable microorganisms include *Corynebacterium ammoniagenes* which are induced to express GMP synthetase/XMP aminase and inosine-guanosine kinase for use in producing IMP and GMP, especially from the nucleotide precursor XMP.

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